convention adopted by Li *et al.*, (1991). Adopting the convention of Li *et al.*, the present inventors have designated helix two as comprising two portions helix 2a and helix 2b.

FIG. 2. Shown are the structural maps of pEG315, pEG916, pEG359, and p154. Boxed arrows and segments indicate genes or functional DNA elements. Designations: pTZ19u = *E. coli* phagemid vector pTZ19u, *cat* = chloramphenicol (Cml) acetyltransferase gene, *ori43* and *ori60* = *B. thuringiensis* plasmid replication origins, *cry1C* = *cry1C* insecticidal crystal protein gene. Restriction site abbreviations: Ag = *Age*I, Asp = *Asp*718, Ba = *Bam*HI, Bb = *Bbu*I, Bg = *Bgl*II, Bln = *Bln*I, , P = *Pst*I, S = *Sal*I, X = *Xho*I. The 1 kb scale refers to only the *cry1C* gene segment. pEG315 gave rise to pEG 1635 and pEG1636, which contain the Arg148Ala and Arg180Ala mutations, respectively. pEG916 gave rise to pEG370, pEG373, and pEG374, which contain the *cry1C.563*, *cry1C.579*, and *cry1C.499* mutations, respectively. These mutants are described in detail in Section 5.

FIG. 3. Shown is the structural map of pEG345. Boxed arrows and segments indicate genes or functional DNA elements. Designations: pTZ19u = *E. coli* phagemid vector pTZ19u, *cat* = Cml acetyltransferase gene, *ori44* = *B. thuringiensis* plasmid replication origin. *cry1C* = *cry1C* insecticidal crystal protein gene. Restriction site abbreviations: Ag = *Age*I, Asp = *Asp*718, Bb = *Bbu*I, Bg = *Bgl*II, E = *Eco*RI, H = *Hin*dIII, Sm = *Sma*I. The 1 kb scale refers to only the *cry1C* gene segment.

FIG. 4. Depicted is a flow chart indicating the mutations contained within the *cry1C* gene encoded by pEG359 and the mutations contained within the *cry1C*.563, *cry1C.579*, and *cry1C.499* genes generated by random mutagenesis.

FIG. 5. Shown is the PCR™-mediated mutagenesis procedure used to generate the mutant *cry1C.499*, *cry1C*.563, and *cry1C.579* genes in strains EG11747, EG11740, and EG11746, respectively. The asterisk denotes mutations incorporated into the *cry1C* gene sequence. Restriction sites abbreviations: Ag=*Age*I, Bb=*Bbu*I, and Bg=*Bgl*II.

FIG. 6. Shown is the alignment of a loop region of 24 related Cry1 proteins.

FIG. 7. Structural maps of the *cryIC*-encoding plasmids pEG348 and pEG348Δ. Boxed arrows and segments indicate genes or functional DNA elements. Designations: pTZ19u = *E. coli* phagemid vector pTZ19u, *tet* = tetracycline resistance gene, *ori60* = *B. thuringiensis* plasmid replication origin, *cryIC* = *cryIC* insecticidal crystal protein gene, IRS = DNA fragment containing the internal resolution site region of transposon Tn*5401*. Restriction site abbreviations: A = *Asp*718, H = *Hin*dIII, Nsi = *Nsi*I, Nsp = *Nsp*I, P = *Pst*I, Sp = *Sph*I.

FIG. 8. Structural maps of the *cryIC*-encoding plasmids pEG1641 and pEG1641Δ. Boxed arrows and segments indicate genes or functional DNA elements. Designations: pTZ19u = *E. coli* phagemid vector pTZ19u, *tet* = tetracycline resistance gene, *ori60* = *B. thuringiensis* plasmid replication origin, *cryIC* = *cryIC* insecticidal crystal protein gene, IRS = DNA fragment containing the internal resolution site region of transposon Tn*5401*. Restriction site abbreviations: A = *Asp*718, H = *Hin*dIII, Nsi = *Nsi*I, Nsp = *Nsp*I, P = *Pst*I, Sp = *Sph*I.

FIG. 9. Shown is the structural map of pEG943. Boxed arrows and segments indicate genes or functional DNA elements. Designations: pTZ19u = *E. coli* phagemid vector pTZ19u, *cat* = Cml acetyltransferase gene, *ori44* = *B. thuringiensis* plasmid replication origin, *cryIC* = *cryIC* insecticidal crystal protein gene. Restriction site abbreviations: Ag = *Age*I, Asp = *Asp*718, Bb = *Bbu*I, Bg = *Bgl*II, E = *Eco*RI, H = *Hin*dIII, Nh = *Nhe*I, Sm = *Sma*I. The 1 kb scale refers to only the *cryIC* gene segment.

FIG. 10. Shown is the overlap extension PCR™ procedure used to generate Cry1C-R148D combinatorial mutants with amino acid substitutions in loop α6-7. The asterisk denotes mutations incorporated into the *cryIC* gene sequence. The PCR™ with the flanking primers H and L yielded a sub-population of fragments encoding mutations in loop α6-7 and lacking the *Nhe*I site derived from the pEG943 template. Restriction site abbreviations: Ag = *Age*I, Asp = *Asp*718, Bb = *Bbu*I, Bg = *Bgl*II, E = *Eco*RI, H = *Hin*dIII, Nh = *Nhe*I, Sm = *Sma*I.

FIG. 11. Shown is the overlap extension PCR™ procedure used to generate Cry1C-R148D combinatorial mutants with amino acid substitutions in loop α5-6. The asterisk denotes mutations incorporated into the *cryIC* gene sequence. The PCR™ with

the flanking primers H and L yielded a sub-population of fragments encoding mutations in loop α5-6 and lacking the *Nhe*I site derived from the pEG943 template. Restriction site abbreviations: Ag = *Age*I, Asp = *Asp*718, Bb = *Bbu*I, Bg = *Bgl*II, E = *Eco*RI, H = *Hin*dIII, Nh = *Nhe*I, Sm = *Sma*I.

4.0 DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

4.1 SOME ADVANTAGES OF THE INVENTION

Mutagenesis experiments with *cry1* genes have failed to identify mutant crystal proteins with improved broad-spectrum insecticidal activity, that is, with improved toxicity towards a range of insect pest species. Since agricultural crops are typically threatened by more than one insect pest species at any given time, desirable mutant crystal proteins are preferably those that exhibit improvements in toxicity towards multiple insect pest species. Previous failures to identify such mutants may be attributed to the choice of sites targeted for mutagenesis. Sites within domain 2 and domain 3 have been the principal targets of previous Cry1 mutagenesis efforts, primarily because these domains are believed to be important for receptor binding and in determining insecticidal specificity (Aronson *et al.*, 1995; Chen *et al.* 1993; de Maagd *et al.*, 1996; Lee *et al.*, 1992; Lee *et al.*, 1995; Lu *et al.*, 1994; Smedley and Ellar, 1996; Smith and Ellar, 1994; Rajamohan *et al.*, 1995; Rajamohan *et al.*, 1996).

In contrast, the present inventors reasoned that the toxicity of Cry1 proteins, and specifically the toxicity of the Cry1C protein, may be improved against a broader array of lepidopteran pests by targeting regions involved in ion channel function rather than regions of the molecule directly involved in receptor interactions, namely domains 2 and 3. Accordingly, the inventors opted to target regions within domain 1 of Cry1C for mutagenesis in the hopes of isolating Cry1C mutants with improved broad spectrum toxicity. Indeed, in the present invention, Cry1C mutants are described that show improved toxicity towards several lepidopteran pests, including *Spodoptera exigua*, *Spodoptera frugiperda*, *Trichoplusia ni*, and *Heliothis virescens*, while maintaining excellent activity against *Plutella xylostella*.